

# User manual Invisorb<sup>®</sup> Spin Plant Mini Kit

For genomic DNA purification from up to 100 mg wet and up to 60 mg of dried plant materia lfrom a wide variety of plant species as well as from food samples of plant origin

REF 1037100x00 STRATEC Molecular GmbH, D-13125 Berlin

# Instruction for the Invisorb® Spin Plant Mini Kit

The Invisorb® Spin Plant Mini Kit has been designed for isolation and purification of total DNA from a wide variety of plant species (fresh, frozen or dried plant material, for instance leaves, roots, fruits or seeds) and also from a wide variety of food sample from plant origin (fresh, frozen or dried material).

The DNA extraction and purification chemistry was intensely tested and validated.

The **Invisorb® Spin Plant Mini Kit** is intended for life science research use only. Prior to using it for other purposes, the user must validate the system in compliance with the applicable law, directives, and regulations.

The kit is neither suitable for isolation of RNA from plant material and food sample from plant origin, as for DNA isolation from cultured or isolated cells, tissue samples or blood samples. Simultaneously isolation of bacterial DNA or DNA from fungi and parasites is not validated.

Trademarks: Invisorb<sup>®</sup>. Registered marks, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

The Invisorb $^{\circ}$  technology is covered by patents and patent applications: US 6,110363, US 6,043,354, US 6,037,465, EP 0880535, WO 9728171, WO 9534569, EP 0765335, DE 19506887, DE 10041825.2, WO 0034463.

Invisorb® is a registered trademark of STRATEC Biomedical AG.

The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

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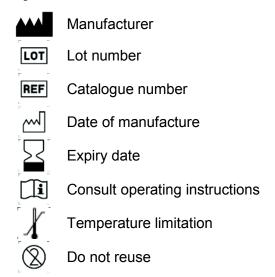
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# Kit contents of the Invisorb® Spin Plant Mini Kit

Store dissolved Proteinase K at –20°C! Store all other kit components at room temperature (RT)!

|                       | 5 DNA preps   | 50 DNA preps  | 250 DNA preps   |
|-----------------------|---|---|---|
| Catalogue No.         | 1037100100  | 1037100200  | 1037100300  |
| Lysis Buffer P        | 2 x 2 ml  | 30 ml   | 120 ml  |
| Binding Buffer A      | 2 x 1 ml<br>(ready to use)  | 4 ml<br>(final volume 15 ml)  | 2 x 9 ml<br>(final volume 2 x 30 ml)  |
| Proteinase K          | for 250 µl<br>working solution  | for 1 ml<br>working solution  | for 5 x 1 ml<br>working solution  |
| Wash Buffer I         | 15 ml<br>(ready to use)   | 30 ml<br>(final volume 60 ml)   | 80 ml<br>(final volume 160 ml)  |
| Wash Buffer II        | 15 ml<br>(ready to use)   | 18 ml<br>(final volume 60 ml)   | 2 x 45 ml<br>(final volume 2 x 150 ml)  |
| Elution Buffer        | 2 ml  | 15 ml   | 60 ml   |
| Prefilter             | 5   | 50  | 5 x 50  |
| Spin Filter           | 5   | 50  | 5 x 50  |
| 2.0 ml Receiver Tubes | 10  | 2 x 50  | 10 x 50   |
| 1.5 ml Receiver Tubes | 5   | 50  | 5 x 50  |
| Manual                | 1   | 1   | 1   |
| Initial steps         | Add 250 µl dd H <sub>2</sub> O to the tube <b>Proteinase K</b> , mix thoroughly and store the tube at -20°C!  Incubate the needed amount of <b>Elution Buffer</b> at 65°C in a Thermomixer. | Add 11 ml 99.7% Isopropanol to the <b>Binding Buffer A.</b> Mix by intensive shaking by inverting for 1 min Shortly before use mix by inverting several times.  Add 30 ml of 96-100% Ethanol to the bottle <b>Wash Buffer I.</b> Add 42 ml of 96-100% Ethanol to the bottle <b>Wash Buffer II</b> , mix thoroughly and store with tightly closed cap.  Add 1 ml dd H <sub>2</sub> O to the tube <b>Proteinase K</b> , mix thoroughly and store the tube at -20°C!  Incubate the needed amount of <b>Elution Buffer at</b> 65°C in a | Add 21 ml 99.7% Isopropanol to each <b>Binding Buffer A.</b> Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times.  Add 80 ml of 96-100% Ethanol to the bottle <b>Wash Buffer I.</b> Add 105 ml of 96-100% Ethanol to each bottle <b>Wash Buffer II.</b> Add 105 ml of 96-100% Ethanol to each bottle <b>Wash Buffer II.</b> Add 105 ml of 96-100% Ethanol to each bottle <b>Wash Buffer II.</b> Add 105 ml of 96-100% Ethanol to each bottle <b>Wash Buffer II.</b> Add 105 ml of 96-100% Ethanol to each bottle <b>Wash Buffer II.</b> Add 105 ml of 96-100% Ethanol to the bottle <b>Wash Buffer II.</b> Add 105 ml of 96-100% Ethanol to the bottle <b>Wash Buffer II.</b> Add 105 ml of 96-100% Ethanol to the bottle <b>Wash Buffer II.</b> Add 105 ml of 96-100% Ethanol to the bottle <b>Wash Buffer II.</b> Add 105 ml of 96-100% Ethanol to the bottle <b>Wash Buffer II.</b> Add 105 ml of 96-100% Ethanol to the bottle <b>Wash Buffer II.</b> Add 105 ml of 96-100% Ethanol to the bottle <b>Wash Buffer II.</b> Add 105 ml of 96-100% Ethanol to the bottle <b>Wash Buffer II.</b> Add 105 ml of 96-100% Ethanol to the bottle <b>Wash Buffer II.</b> Add 105 ml of 96-100% Ethanol to the bottle <b>Wash Buffer II.</b> Add 105 ml of 96-100% Ethanol to the bottle <b>Wash Buffer II.</b> Add 105 ml of 96-100% Ethanol to the bottle <b>Wash Buffer II.</b> Add 105 ml of 96-100% Ethanol to the bottle <b>Wash Buffer II.</b> Add 105 ml of 96-100% Ethanol to the bottle <b>Wash Buffer II.</b> Add 105 ml of 96-100% Ethanol to the bottle <b>Wash Buffer II.</b> Add 105 ml of 96-100% Ethanol to the bottle <b>Wash Buffer II.</b> Add 105 ml of 96-100% Ethanol to the bottle <b>Wash Buffer II.</b> Add 105 ml of 96-100% Ethanol to the bottle <b>Wash Buffer II.</b> Add 105 ml of 96-100% Ethanol to the bottle <b>Wash Buffer II.</b> Add 105 ml of 96-100% Ethanol to the bottle <b>Wash Buffer II.</b> Add 105 ml of 96-100% Ethanol to the bottle <b>Wash Buffer II.</b> Add 105 ml of 96-100% Ethanol to the bottle <b>Wash Buffer II.</b> Add 105 ml of 96-100% Ethanol to the bottle <b>Wash Buffer II.</b> Add 105 ml of 96-100% Ethanol to the bottle <b>Wash Buffer II</b> |

# **Symbols**



## **Storage**

All buffers and kit components of the **Invisorb**<sup>®</sup> **Spin Plant Mini Kit** should be stored at room temperature (RT) and are stable for 12 months under these conditions.

Room temperature (RT) is defined as range from 15 - 30°C.

Dissolved **Proteinase K** stored at –20°C is stable for 12 months, but repeated freezing and thawing should be avoided. Aliquotation and storage at – 20°C is recommended.

**Wash Buffer** charged with ethanol should be appropriately sealed and stored at room temperature.

If there are any precipitates within the provided solutions dissolve these precipitates by carefully warming up to room temperature (up to 30°C).

# Quality control and product warranty

STRATEC Molecular warrants the correct function of the **Invisorb**<sup>®</sup> **Spin Plant Mini Kit** for applications as described in this manual. Purchaser must determine the suitability of the Product for its particular use. Should any Product fail to perform the applications as described in the manual, STRATEC Molecular will check the lot and if STRATEC Molecular investigates a problem in the lot, STRATEC Molecular will replace the Product free of charge.

STRATEC Molecular reserves the right to change, alter, or modify any Product to enhance its performance and design at any time.

In accordance with STRATEC Molecular's ISO 9001-2000 and ISO EN 13485 certified Quality Management System the performance of all components of the Invisorb® Spin Plant Mini Kit

have been tested separately against predetermined specifications routinely on lot-to-lot to ensure consistent product quality.

If you have any questions or problems regarding any aspects of **Invisorb® Spin Plant Mini Kit** or other STRATEC Molecular products, please do not hesitate to contact us. A copy of STRATEC Molecular's terms and conditions can be obtained upon request or are presented at the STRATEC Molecular webpage.

For technical support or further information please contact: from Germany +49-(0)30-9489-2901/ 2910 from abroad +49-(0)30-9489-2907 or contact your local distributor.

#### Intended use

The Invisorb® Spin Plant Mini Kit is the ideal tools for a rapid and efficient isolation of high quality genomic DNA from up to 100 mg of a wide variety of plant species (fresh, frozen or dried plant material, for instance leaves, roots, fruits or seeds) and from food sample from plant origin.

The protocols for the isolation and all buffers are optimized for a high yield as well as a high purity. All hands on steps are reduced to a minimum.

For reproducible and high yields appropriate sample storage is essential.

THE PRODUCT IS INDENTED FOR USE BY PROFESSIONAL USERS ONLY, SUCH AS TECHNICIANS, PHYSICIANS AND BIOLOGISTS TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES. It is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modification of DNA followed by signal detection or amplification. Any results generated using the sample preparation procedure in conjunction with any downstream assay should be interpreted with regard to other laboratory findings.

To minimize irregularities in your results, adequate controls for downstream applications should be used.

#### **Product use limitation**

The kit is neither suitable for isolation of RNA from plant material, as for DNA isolation from cultured or isolated cells, tissue samples or blood samples. The isolation of simultaneously isolation of bacterial DNA or DNA from fungi and parasites is not validated.

The included chemicals are only useable once.

Differing of starting material or flow trace may lead to inoperability; therefore neither a warranty nor guarantee in this case will be given, neither implied nor express.

The user is responsible to validate the performance of the STRATEC Molecular Product for any particular use. STRATEC Molecular does not provide for validation of performance characteristics of the Product with respect to specific applications. STRATEC Molecular Products may be used e.g.in clinical diagnostic laboratory systems conditioned upon the complete diagnostic system of the laboratory the laboratory has been validated pursuant to CLIA' 88 regulations in the U.S. or equivalents in other countries.

All Products sold by STRATEC Molecular are subject to extensive quality control procedures (according to ISO 9001-2000 and ISO EN 13485) and are warranted to perform as described herein. Any problems, incidents or defects shall be reported to STRATEC Molecular immediately upon detection thereof.

The chemicals and the plastic parts are for laboratory use only; they must be stored in the laboratory and must not be used for purposes other than intended.

The Product with its contents is unfit for consumption.

# **Safety information**

When and while working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles!

Avoid skin contact! Adhere to the legal requirements for working with biological material! For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at <a href="www.stratec.com">www.stratec.com</a> for each STRATEC Molecular Product and its components. If buffer bottles are damaged or leaking, WEAR GLOVES, AND PROTECTIVE GOGGLES when discarding the bottles in order to avoid any injuries.

STRATEC Molecular has not tested the liquid waste generated by the **Invisorb® Spin Plant Mini Kit** procedure for residual infectious materials. Contamination of the liquid waste with residual infectious materials is highly unlikely, but cannot be excluded completely. Therefore, liquid waste must be considered infectious and be handled and discarded according to local safety regulations.

European Community risk and safety phrases for the components of the **Invisorb**<sup>®</sup> **Spin Plant Mini Kit** to which they apply, are listed below as follows:

Wash Buffer I contains guanidine thiocyanate which is an irritant.

#### Lysis Buffer P



danger H319 P305-351-338

#### Wash Buffer I



warning

H302-312-332-412 EUH032 P273

#### Proteinase K



danger

H315-319-334-335 P280-305-351-338-310-405

**H319:** Causes serious eye irritation.

**H315:** Causes skin irritation.

**H334:** May cause allergy or asthma symptoms or breathing difficulties if inhaled.

**H335:** May cause respiratory irritation.

H302: Harmful if swallowed.
H312: Harmful in contact with skin.

**H332:** Harmful if inhaled.

**H412:** Harmful to aquatic life with long lasting effects. **EUH032:** Contact with acids liberates very toxic gas.

P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if

present and easy to do. Continue rinsing.

**P280:** Wear protective gloves/protective clothing/eye protection/face protection.

P310: Immediately call a POISON CENTER or doctor/physician.

P405: Store locked up.

**P273:** Avoid release to the environment.

#### **Emergency medical information can be obtained 24 hours a day from infotrac:**

outside of USA: 1 - 352 - 323 - 3500in USA: 1 - 800 - 535 - 5053

# Product characteristic of the Invisorb® Spin Plant Mini Kit

| Starting material/   | Yield   | Time for preparation               | Ratio  |
|--|---|------------------------------------|--|
| up to 100 mg of plant material<br>up to 60 mg of dried plant<br>material | up to 50 µg depends or amount and kind of starting material | about 20<br>minutes after<br>lysis | A <sub>260</sub> : A <sub>280</sub><br>1.6 – 2.0 |

The Invisorb® Spin Plant Mini Kit provide a very efficient procedure for isolation of high quality genomic DNA from a wide variety of plant species

The **Invisorb**<sup>®</sup> **Spin Plant Mini Kit** combine the lysis of starting material with the very efficient binding of genomic DNA onto a spin filter surface without chaotropic ions.

The isolation protocol as well as all buffers is optimized to provide high yield and purity of the isolated genomic DNA. The "hands-on time" necessary for the whole procedure is reduced to minimum.

The purification procedure is rapid and requires neither phenol / chloroform extraction nor alcohol precipitation, and requires minimal interaction by the user.

Due to the high purity, the isolated genomic DNA is ready to use for a broad panel of downstream applications (see below) or can be stored at -20°C for subsequent use.

#### **Downstream Application:**

- PCR \*)
- o RFLP-Analysis
- Restriction Enzyme Digestion
- Southern Blot Analysis
- Sequencing
- Cloning

To purify genomic DNA in 96 format STRATEC Molecular offers the Invisorb® DNA Plant HTS 96 Kit for use in a centrifuge and on common laboratory automated workstations. Furthermore STRATEC Molecular offers the InviMag® Plant DNA Kits for DNA isolation using magnetic beads for use on a KingFisher® mI or a KingFisher® 96

For further information please contact: Tel.: +49 (0) 30 9489 2901, 2910 in Germany and from foreign countries Tel.: +49 (0) 30 9489 2907 or your local distributor.

<sup>\*)</sup> The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

## Principle and procedure

The Invisorb® Spin Plant Mini Kit procedure comprises following steps:

- lysis of sample material
- binding the genomic DNA to the membrane
- washing and elimination of ethanol
- elution of genomic DNA

After lysis the DNA binds to the membrane, contaminations and enzyme inhibitors are efficiently removed during the following wash steps and highly purified DNA is eluted in Elution Buffer or water.

## Sampling and storage of starting material

Harvested plant samples/ food sample from plant origin can be stored at room temperature for 2 – 3 hours, for short time storage (up to one week) samples may be stored at 4°C. For long term storage, we recommend freezing samples at –20°C or –80°C. Multiple thawing and freezing before isolating the DNA should be avoided.

# Yield and quality of genomic DNA

The amount of purified DNA in the **Invisorb**<sup>®</sup> **Spin Plant Mini Kit** procedure from plant material depends on sample source, transport conditions, storage and age of the sample. Yield and quality of isolated genomic DNA is suitable for any detection system.

## Important notes

## Important points before starting a protocol

Immediately upon receipt of the Product, inspect the Product and its components as well as the package for any apparent damages, correct quantities and quality. If there are any unconformities you have to notify STRATEC Molecular in writing with immediate effect upon inspection thereof. If buffer bottles are damaged, contact the STRATEC Molecular Technical Services or your local distributor. In case of liquid spillage, refer to "Safety Information" (see page 7). Do not use damaged kit components, since their use may lead to poor kit performance.

- Always change pipet tips between liquid transfers. To avoid cross-contamination, we recommend the use of aerosol-barrier pipet tips.
- All centrifugation steps are carried out at room temperature.
- When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.
- Discard gloves if they become contaminated.
- Do not combine components of different kits unless the lot numbers are identical.
- Avoid microbial contamination of the kit reagents.
- To minimize the risk of infections from potentially infectious material, we recommend working under laminar air-flow until the samples are lysed.
- This kit should only be used by trained personnel.

# Important indications

- 1. Process only as much plant samples as the microcentrifuge allows to process.
- 2. Plant sample and buffers should be thoroughly mixed and should have room temperature
- 3. The elution can be done by using lower amount of **Elution Buffer**. This may result in a higher concentration of DNA. But pay attention that minimum volume for elution is 50 μl, but this will reduce the yield.

- **4.** The eluted DNA volume can be lower than the added **Elution Buffer** volume.
- **Elution Buffer** should be preheated to 65 °C.
- The **Elution Buffer** doesn't contain EDTA.
- 7. The yield can be increased, if the incubation time with preheated **Elution Buffer** will be prolonged.

# Preparing reagents and buffers of the Invisorb® Spin Plant Mini Kit

- 1. Adjust the thermo mixer to 65°C.
- 2. Warm up the needed amount of Elution Buffer to 65°C.
- **3.** Label the needed amount of Spin Filter.
- **4.** Label the needed amount of reaction tubes.
- 5. Add the needed  $\mu I ddH_2O$  to reaction tube with **Proteinase K** (see below). Vortex for 5 s.
- 6. Add the needed amount of ethanol to the Wash Buffers.

# Reagents and equipment to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at www.stratec.com under each STRATEC Molecular kit and kit component.

- Microcentrifuge
- Thermomixer (for 65°C)
- Reaction tubes (1.5 ml)
- dd H<sub>2</sub>O

- 96 100 % ethanol
- RNase A (10 mg/ ml optional)
- Isopropanol\*

\*The Invisorb® Spin Plant Mini Kit is validated with 2-Propanol; Rotipuran >99.7%, p.a., ACS, ISO (Order no. 6752) from Carl Roth

#### **Carl Roth**

**Applichem** Sigma 2-Propanol 2-Propanol für die Molekularbiologie 2-Propanol Rotipuran >99.7%, p.a., ACS, ISO Order no. A3928 Order no. 6752

# Order no. 59304-1L-F

#### **5 DNA extractions:**

Add 250 µl dd H<sub>2</sub>O to the tube **Proteinase K**, mix thoroughly and store the tube at -20°C! Incubate the needed amount of Elution Buffer at 65°C in a Thermomixer.

#### 50 DNA extractions:

Add 11 ml 99.7% Isopropanol to the Binding Buffer A.

Add 30 ml of 96-100% Ethanol to the bottle Wash Buffer I.

Add 42 ml of 96-100% Ethanol to the bottle Wash Buffer II, mix thoroughly and store with tightly

Add 1 ml dd H<sub>2</sub>O to the tube **Proteinase K**, mix thoroughly and store the tube at -20°C! Incubate the needed amount of **Elution Buffer** at 65°C in a Thermo mixer.

#### 250 DNA extractions:

Add 21 ml 99.7% Isopropanol to each Binding Buffer A.

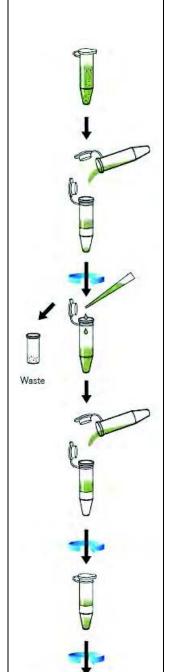
Add 80 ml of 96-100% Ethanol to the bottle Wash Buffer I.

Add 105 ml of 96-100% Ethanol to each bottle Wash Buffer II, mix thoroughly and store with tightly

Add 1 ml dd H<sub>2</sub>O to the tube **Proteinase K**, mix thoroughly and store the tube at-20°C! Incubate the needed amount of **Elution Buffer** at 65°C in a Thermo mixer.

<sup>\*</sup> Possible suppliers for Isopropanol:

# Scheme of the Invisorb® Spin Plant Mini Kit



genomic DNA

#### Please read protocols prior the start of the preparation carefully

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Homogenize about 60 mg of starting material by a pestle under liquid N2

Transfer homogenized plant material into a 1.5 ml reaction tube. Add 400  $\mu$ l **Lysis Buffer P** and 20  $\mu$ l **Proteinase K**, vortex briefly. Incubation at 65°C for 30 min

Transfer of Lysis Solution onto the Prefilter. Centrifuge for 1 min at 11.100 x g (11.000 rpm) Discard the Prefilter

Add 200 µl of Binding Buffer A (follow preparing instructions) and vortex thoroughly

Transfer the suspension onto the Spin Filter.

Incubate for 1 min.

Centrifuge at 11.100 x g (11.000 rpm) for 2 min. Discard the filtrate

Add 550 µl Wash Buffer I.

Centrifuge at 11.100 x g (11.000 rpm) for 1min.

Discard the filtrate

Place the Spin Filter again into the 2.0 ml Receiver Tube.

Add 550  $\mu$ l **Wash Buffer II** and centrifuge at 11.100 x g (11.000 rpm) for 1min. Discard the filtrate,

Repeat the washing step once again.

Discard the filtrate and centrifuge for 4 min at 11.100 x g (11.000 rpm)

Place the Spin Filter into a new 1.5 ml Receiver Tube and add 100  $\mu$ l of the prewarmed **Elution Buffer**.

Incubate for 3 min. Centrifuge for 1 min at 11.100 x g (11.000 rpm).

Discard Spin Filter

Close the 1.5 ml Receiver Tube and store the DNA sample at 4 °C

# Protocol 1: DNA extraction from fresh or dried plant material and from food of plant origin (fresh, frozen or dried material)

Please read the instructions carefully and conduct the prepared procedure.

Attention: Please be aware, that you have to prepare the Binding Buffer A – see instruction page: 8

Important Transfer the needed amount of Elution Buffer into 2.0 ml Receiver

Tube (not included in the kit) and place the tube a 65°C.

#### 1. Homogenization of the starting material

Homogenize about 60 mg of starting material by a pestle under liquid  $N_2$ . Commercially available equipment for homogenization also can be used.

**Note:** Use 120-180 mg of starting material if extraction from material containing more water

(fruits, algae).

#### 2. Lysis of the starting material

Transfer the "plant powder" into a 1.5 ml reaction tube. Add 400  $\mu$ l Lysis Buffer P and 20  $\mu$ l Proteinase K and vortex briefly. Incubation at 65°C for 30 min or longer (incubation in a thermo mixer under continuous shaking is recommended). During incubation place the Prefilter into a 2.0 ml Receiver Tube.

#### 3. Filtration of Lysis Solution and realizing optimum binding conditions

Transfer of Lysis Solution onto the Prefilter. Centrifuge for 1 min at 11.100 x g (11.000 rpm) Discard the Prefilter.

Note: To remove RNA (if it is necessary) from the sample add 40 μl of RNase A to the filtrate

(10 mg/ml), vortex briefly and incubate for 5 min at room temperature.

Add 200 µl of **Binding Buffer A** and vortex thoroughly.

#### 4. DNA Binding

Place a the Spin Filter into a 2.0 ml Receiver Tube. Transfer the suspension onto the Spin Filter and incubate for 1 min. Centrifuge at 11.100 x g (11.000 rpm) for 2 min. Discard the filtrate and place the Spin Filter again into the 2.0 ml Receiver Tube.

#### 5. Washing I

Add 550 µl **Wash Buffer I** and centrifuge at 11.100 x g (11.000 rpm) for 1min. Discard the filtrate, place the Spin Filter again into the 2.0 ml Receiver Tube.

#### 6. Washing II

Add 550  $\mu$ l **Wash Buffer II** and centrifuge at 11.100 x g (11.000 rpm) for 1min. Discard the filtrate, place the Spin Filter again into the Receiver Tube and repeat the washing step once again. Finally discard the filtrate and centrifuge for 4 min at 11.100 x g (11.000 rpm) to remove residual ethanol.

#### 7. Elution of the DNA

Place the Spin Filter into a new 1.5 ml Receiver Tube and add 100  $\mu$ l of the prewarmed Elution Buffer. Incubate for 3 min. Centrifuge for 1 min at 11.100 x g (11.000 rpm).

Note:

The DNA can also be eluted with a lower or a higher volume of Elution Buffer (depends on the expected yield of genomic DNA). But pay attention, that minimum volume for the elution is 50  $\mu$ l. If quite large amount of DNA is expected, the volume of elution can be increased (100-200  $\mu$ l).

To maximize the final yield we recommend a second elution step with the equal volume of Elution Buffer.

# **Troubleshooting**

| Problem  | Cause   | Comments and suggestions   |
|--|---|--|
| clogged Spin Filter  | insufficient lysis and/or too<br>much starting material         | increase lysis time increase centrifugation speed or time reduce amount of starting material   |
| low amount of extracted DNA  | insufficient lysis incomplete elution                           | increase lysis time reduce amount of starting material overloading of Spin Filter reduces yield prolong the incubation time with <b>Elution Buffer</b> to 5-10 min or repeat elution step once again   |
|  | insufficient mixing with Binding Buffer A                       | take higher volume of <b>Elution Buffer</b> Mix sample with <b>Binding Buffer A</b> by pipetting or by vortexing prior to transfer the sample onto the Spin Filter   |
| low concentration of extracted DNA                                     | too much Elution Buffer   | elute the DNA with lower volume of <b>Elution Buffer</b>   |
| degraded or sheared DNA  | incorrect storage of starting material old material             | ensure that the starting material is frozen immediately in liquid $N_2$ or in minimum at $-20^{\circ}\text{C}$ and is stored continuously at $-80^{\circ}\text{C}$ avoid thawing and freezing of the material. old material often contains degraded DNA. |
| RNA contaminations of extracted DNA.                                   | to much RNA   | RNAse A digestion  |
| genomic DNA does<br>not perform well in<br>downstream-<br>applications | ethanol carryover during elution  Salt carryover during elution | increase time for removing of ethanol. ensure that <b>Wash Buffer</b> is at room temperature. check up <b>Wash Buffer</b> for salt precipitates. If there are any precipitates, solve these precipitates by careful warming.                             |

## **Appendix**

#### General notes on handling DNA

#### **Nature of DNA**

The length and delicate physical nature of DNA requires careful handling to avoid damage due to shearing and enzymatic degradation. Other conditions that affect the integrity and stability of DNA include acidic and alkaline environments, high temperature, and UV irradiation. Careful isolation and handling of high molecular weight DNA is necessary to ensure compatibility with various downstream applications. Damaged DNA could perform poorly in applications such as genomic Southern blotting and long-template PCR.

#### Storage of DNA

A working stock of DNA can be stored at  $2-4^{\circ}$ C for several weeks. For long term storage DNA should be stored at -20°C, but storing at  $-20^{\circ}$ C can cause shearing, particularly if the DNA is exposed to repeated freeze-thaw cycles.

Note that the solution in which the nucleic acid is eluted in will affect it's stability during storage. Pure water lacks buffering capacity and an acidic pH may lead to acid hydrolysis. Tris or Tris-EDTA buffer contains sufficient buffering capacity to prevent acid hydrolysis.

#### Drying, dissolving and pipetting DNA

Avoid over drying genomic DNA after ethanol precipitation. It is better to let it air dry than to use a vacuum, although vacuum drying can be used with caution.

Avoid vigorous pipetting. Pipetting genomic DNA through small tip openings causes shearing or nicking. One way to decrease shearing of genomic DNA is to use special tips that have wide openings designed for pipetting genomic DNA.

#### **DNA Yield**

The amount of purified DNA from the plant material depends on sample source, transport conditions, storage and age of the sample.

# **Ordering information**

| Product   | Package size       | Catalogue No. |  |  |  |  |
|---|--------------------|---------------|--|--|--|--|
| Invisorb <sup>®</sup> Spin Plant Mini Kit                           | 5 preparations     | 1037100100    |  |  |  |  |
| Invisorb <sup>®</sup> Spin Plant Mini Kit                           | 50 preparations    | 1037100200    |  |  |  |  |
| Invisorb <sup>®</sup> Spin Plant Mini Kit                           | 250 preparations   | 1037100300    |  |  |  |  |
| Single components for the Invisorb <sup>®</sup> Spin Plant Mini Kit |                    |               |  |  |  |  |
| Lysis Buffer P  | 30 ml              | 1037101200    |  |  |  |  |
| Binding Buffer A  | 15 ml              | 1037102800    |  |  |  |  |
| Wash Buffer I (add 30 ml)   | 30 ml              | 1037103300    |  |  |  |  |
| Wash Buffer II (add 42 ml)  | 18 ml              | 1037103400    |  |  |  |  |
| Elution Buffer  | 15 ml              | 1037104000    |  |  |  |  |
|   |                    |               |  |  |  |  |
| Related products  |                    |               |  |  |  |  |
| Invisorb® DNA Plant 96 HTS/ C                                       | 2x96 preparations  | 7037300200    |  |  |  |  |
| Invisorb® DNA Plant 96 HTS/ C                                       | 4x96 preparations  | 7037300300    |  |  |  |  |
| Invisorb <sup>®</sup> DNA Plant 96 HTS/ C                           | 24x96 preparations | 7037300400    |  |  |  |  |
|   |                    |               |  |  |  |  |
| InviMag <sup>®</sup> Plant DNA Mini Kit/ KFm                        | • •                | 2437110100    |  |  |  |  |
| InviMag® Plant DNA Mini Kit/ KFm                                    | 75 preparations    | 2437110200    |  |  |  |  |
| InviMag® Plant DNA Mini Kit/ KF96                                   | 1x96 preparations  | 7437300100    |  |  |  |  |
| InviMag <sup>®</sup> Plant DNA Mini Kit/ KF96                       | • •                | 7437300200    |  |  |  |  |
| <u>-</u>  | • •                |               |  |  |  |  |

# Possible suppliers for Isopropanol

Carl RothApplichemSigma2-Propanol2-Propanol für die Molekularbiologie2-PropanolRotipuran >99.7%, p.a., ACS, ISOOrder no. A3928Order no. 59304-1L-F



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